

about 200 years ago, retreated up bay. Previous sidescan sonar cruises have shown some ice gouging farther up the fjord, but the wide-spread nature of the gouges in the lowermost portions of Glacier Bay, as shown by the multibeam image between Sitakaday Narrows and the fjord entrance, were unexpected. Massive icebergs with drafts to 100m calved repeatedly from the glacier, as it retreated up the fjord. The dominant gouge orientation, roughly parallel to the fjord axis, suggests the strong tidal currents of up to seven knots through Sitakaday Narrows were responsible for driving the icebergs keels across the seabed. The gouges remain unburied in this environment of high sedimentation because even though the glaciers have retreated more than 80 km up fjord from Sitakaday Narrows, the amount of sediment presently reaching the ice gouges is largely restricted to local runoff and plankton debris. In addition, the strong tidal currents through Sitakaday Narrows, effectively keep the ice-gouged fjord floor scoured clean of fine sediment. This multibeam imagery is being used in our joint study of physical and biological characteristics of benthic habitats in Glacier Bay and results will be applied to fisheries problems in south east Alaska, especially to Marine Protected Areas.

#### B12C-0146 1330h POSTER

#### A Quantitative Look at Sources and Fates of Inorganic Nutrients at Skorradalur, Iceland

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Bedrock chemistry plays an important role in shaping plant communities in areas where roots contact bedrock or water that contains nutrients derived from the bedrock. Where bedrock and soil lack certain nutrients plants must rely more on atmospheric inputs or efficient recycling-mechanisms. At Skorradalur, Iceland, the bedrock contains little K, and plant growth is particularly sensitive to fluctuations in atmospheric inputs of K.

This study examines the sources and fates of K, Mg, and Ca at 5 small catchments underlain by tholeiitic basalt at Skorradalur, Iceland. The bedrock is overlain by up to 500 cm of basaltic till which was deposited when glaciers retreated from this area between 7000 and 10000 years ago. Catchment vegetation varies from mosses and lichens to birches to mixed conifers. Samples of stream water, throughfall, precipitation, soil, and rock were collected at varying intervals between 1995 and 1998 (Moulton, West, and Berner, 2000). All samples were analyzed for major element chemistry.

Sources of nutrients were calculated as percentage of nutrients derived from atmospheric inputs and bedrock for the summer and winter seasons. Nearly all of the K originates from atmospheric input throughout the year. Relatively more Mg and Ca are supplied from the atmosphere during the summer (about 60 and 50 percent, respectively) than during the winter (about 50 and 25 percent); the remainder is supplied from bedrock.

The fate of K, Mg, and Ca within the catchments was traced using calculations of nutrient fluxes from basalt weathering, atmospheric inputs, biomass storage, and soil storage in secondary minerals. Biomass storage in living and dead tissue accounts for about 60 percent of the total K input, less than 2 percent is stored in soil, and the remainder exits in stream water. Biomass and soil storage account for about 6 and 13 percent, respectively, of the Mg and Ca inputs. These results show that plants at Skorradalur rely almost exclusively on atmospheric inputs and biomass recycling to provide K for growth.

Moulton, K.L., West, J., and Berner, R.A., 2000, Solute Flux and Mineral Mass Balance Approaches to the Quantification of Plant Effects on Silicate Weathering, *American Journal of Science*, v. 300, p. 539-570.

#### B12D MC: Hall D Monday 1330h

#### Assessment and Prevention of Interplanetary Biocontamination (joint with P)

**Presiding:** D Thomas, University of Hawaii

#### B12D-0147 1330h POSTER

#### Developing Planetary Protection Technology: Microbial Diversity of the Mars Orbiter Odyssey and the Spacecraft Assembly and Encapsulation Facility II

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Sampling the surfaces of both spacecraft and their clean-room assembly facilities is crucial in monitoring the microbial burden associated with these pseudo-sterile, oligotrophic environments. Here, we present the results of a study in which several surface samples, retrieved from both the Mars Odyssey Spacecraft and the Kennedy Space Center (KSC) Spacecraft Assembly and Encapsulation Facility II (SAEF-II), were processed and evaluated by both molecular and traditional culture-based methods for microbial diversity. The findings of this study improve our current understanding of the microbial community structure, diversity, and dispersal in a spacecraft assembly facility, as well as physically associated with co-located spacecraft. Surfaces of 25 cm<sup>2</sup> (spacecraft) or 0.4 m<sup>2</sup> (SAEF-II) were swabbed or wiped, respectively, and were examined for total heterotrophic aerobes and spore-formers. Samples were further subjected to nucleic acid extraction, and 16S rDNA fragments were PCR amplified with eubacterial biased universal primers and cloned. Approximately 30 isolates grown by traditional culture-based techniques were included for 16S rDNA sequencing. For the most part, the population dynamics remained consistent when compared between the spacecraft and assembly facility libraries. Predominant microbes, as indicated by molecular methods, included members of the genera *Variovorax* and *Aquaspirillum*. Members of the *Mesorhizobium*, *Bradyrhizobium*, *Enterococcus*, *Ralstonia*, and *Bacillus* genera were also found to span the various libraries but in less abundance. Traditional culture-based techniques validated the presence of *Bacillus* and *Ralstonia*, while illuminating a larger diversity in revealing the presence of *Staphylococcus*, *Comamonas*, *Microbacterium*, and *Actinomyces*. The bulk of these findings make sense, since species of *Ralstonia*, *Rhizobium*, *Variovorax*, and *Bacillus* are known to frequently inhabit rhizospheric environments, like that surrounding the KSC facility, and can thus easily gain way into the clean-room via mixing of surrounding air upon human entry and exit. Also, *Aquaspirillum* species are known to inhabit freshwater and brackish ponds, much like the ones at KSC. Of particular interest to the authors was the presence of *Nicotiana tabacum* chloroplast 16S rDNA in one of the spacecraft samples. The lack of tobacco farming anywhere in the vicinity of the KSC facility leads the authors to speculate that this contamination arose from human contact with the spacecraft, specifically after handling a cigarette, cigar, or other tobacco products. Overall, our findings validate the purpose of planetary protection activities, which improve our knowledge of the types of microbial burden present, and their methods of entry into spacecraft assembly facilities.

#### B12D-0148 1330h POSTER

#### Developing Planetary Protection Technology: Microbial Diversity and Radiation Resistance of Microorganisms in a Spacecraft Assembly Facility.

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Europa has attracted much attention as evidence suggests the presence of a liquid ocean beneath this Jupiter moon's frozen crust. Such an environment might be conducive to the origins of life. Since robotic exploration of Europa is being planned, it becomes crucial to prepare for bio-burden reduction of hardware assembled for Europa missions to avoid contamination of Europa's pristine environment. In this study, we examined the microbial diversity of samples collected from two flight-ready circuit boards and their assembly facility. Also, because Jupiter's strong radiation environment may be able to reduce the viable microbial contamination on flight components, we have also studied the effects of radiation on microbial communities found to be associated with the space-flight hardware and/or present in the assembly facility. Surface samples thought to be representative of considerable human contact were collected from two circuit boards and various locations within the assembly facility using polyester swabs (swab samples). Likewise, sterile wipes were used to sample a shelf above the workstation where the circuit boards were assembled and the floor of the facility (wipe samples). The swab and wipe samples were pooled separately and divided into two halves, one of which was irradiated with 1Mrad gamma radiation for 5.5 hours, the other was not irradiated. About 1.2x10<sup>4</sup> and 6x10<sup>4</sup> CFUs/m<sup>2</sup> cultivable microbes were detected in the swab and wipe samples, respectively. Radiation proved effective in inhibiting the growth of most microbes. Further characterization of the bacterial colonies observed in the irradiated swab and wipe samples is necessary to determine the degree of the radiation resistance. The 16S rDNA sequence analysis of the cultivable microbes indicated that the assembly facility consists mostly of the members of actinobacteria, corynebacteria and pseudomonads. However, the swab samples that include the circuit boards were predominantly populated with *Bacillus* and *Staphylococcus*. Molecular microbial diversity was also studied by cloning the 16S rDNA PCR fragment from the samples. The non-irradiated swab samples were largely populated by species of *Exiguobacter* and *Bacillus* whereas the irradiated swab samples were dominated by *Bacillus* and *E. coli*. Radiation damage of microorganisms was also investigated by epifluorescence microscopy. In summary, our study has shown that gamma radiation can inhibit the growth of most of the cultivable microbes, but preliminary results suggest that radiation such as this has little adverse effect on the DNA molecules of these microorganisms.

#### B12D-0149 1330h POSTER

#### Developing Planetary Protection Technology: Recurrence of Hydrogen Peroxide Resistant Microbes from Spacecraft Assembly Facilities

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Hydrogen peroxide vapor is currently the sterilant-of-choice for flight hardware because it is a low-heat sterilization process suitable for use with various spacecraft components. Hydrogen peroxide is a strong oxidizing agent that produces hydroxyl free radicals ( $\cdot\text{OH}$ ) which attack essential cell components, including lipids, proteins, and DNA. Planetary protection research efforts at the Jet Propulsion Laboratory (JPL) are focused on developing cleaning and sterilization technologies for spacecraft preparation prior to launch. These efforts include research to assess the microbial diversity of spacecraft assembly areas and any extreme characteristics these microbes might possess.

Previous studies have shown that some heat-tolerant *Bacillus* species isolated from the JPL Spacecraft Assembly Facility (SAF) are resistant to recommended hydrogen peroxide vapor sterilization exposures. A *Bacillus* species, which was related to a hydrogen peroxide resistant strain, was repeatedly isolated from various locations in the JPL-SAF. This species was found in both unclassified (entrance floors, anteroom, and air-lock) and classified (class 100K) (floors, cabinet tops, and air) areas. The phylogenetic affiliation of these strains was carried out using biochemical tests and 16S rDNA sequencing. The 16S rDNA analysis showed >99% sequence similarity to *Bacillus pumilus*. In order to understand the epidemiology of these strains, a more highly evolved gene (topoisomerase II  $\beta$ -subunit, *gyrB*) was also sequenced. Among

4 clades, one cluster, comprised of 3 strains isolated from the air-lock area, tightly aligned with the *B. pumilus* ATCC 7061 type strain (97%). The *gyrB* sequence similarity of this clade was only 91% with the 3 other clades. The genetic relatedness of these strains, as per pulse field gel electrophoresis patterns, will be presented.

The vegetative cells and spores of a number of isolates were tested for their hydrogen peroxide resistance. Cells and spores were separately treated with 5% liquid hydrogen peroxide. After 60 minutes of exposure, the samples were diluted in tryptic soy broth and incubated at 32°C. Vegetative cells of one of the isolates, FO-036b, were the only cells to survive the exposure to hydrogen peroxide. In contrast, spores of several of the isolates survived exposure to hydrogen peroxide. Spores of these isolates do not appear to have any obvious morphological changes. We are in the process of analyzing these hydrogen peroxide resistant spores and comparing them to spores of microbes that are not as hydrogen peroxide resistant. The impact and implications of the identification and recurrence of these hydrogen peroxide microbes, and their spores, will be discussed.

## B12D-0150 1330h POSTER

### Developing Planetary Protection Technologies: Isolation and Characterization of Novel Microbes from a Spacecraft Assembly Facility

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Systematic detection and classification of cultivable microbes present in the Spacecraft Assembly Facility (SAF) at the Jet Propulsion Laboratory (JPL) were carried out using classical microbial phylogeny and advanced molecular microbial ecology methods. This work is being carried out to understand the microbial diversity in an assembly facility that could potentially contaminate an assembled spacecraft. Contamination of spacecraft surfaces with terrestrial microbes could compromise the interpretation of results from in-situ life detection studies or sample return missions. Fifty samples were collected from various locations of JPL-SAF whose air circulation and humidity are controlled and maintained to the cleanliness of a class 100K clean-room. Sampling locations included both unclassified (entrance floors, shoe-cleaner, air-shower, ante-room, and air-lock), and classified (clean-room floors, clean-room tables and cabinet tops) areas. All samples were analyzed for the incidence of aerobic spore-formers (using Tryptic Soy Agar) and for total aerobic heterotrophs (using R2 Agar). Spore-former incidence ranged from 0 to  $3 \times 10^{-1}$  CFU/25cm<sup>2</sup> in the unclassified area whereas only 2 out of 25 samples collected in the unclassified areas contained spore-formers. However, the counts of total heterotrophs were about 100 times higher when compared to the spore-formers in the unclassified area and 9 out of 25 samples collected in the classified area contained a range of heterotrophs from  $5 \times 10^1$  to  $4 \times 10^2$  CFU/25cm<sup>2</sup>. Representatives of the spore-formers (31 strains) and total heterotrophs (40 strains) were identified by 16S rDNA sequence analysis to determine their phylogenetic relatedness. The spore-formers clustered to 8 known *Bacillus* and *Paenibacillus* species and 6 strains were identified as novel species of the genera *Bacillus* (2), *Paenibacillus* (2), *Ureibacillus* (1), and one new genus. Among the forty heterotrophs, 5 clusters were tightly affiliated with genera, such as *Bacillus* (5), *Staphylococcus* (2), and members of the families actinomycetales (3), streptomycetes (1), and micrococciaceae (10). However, 19 of the strains isolated clustered to a very distinct clade and formed a relatively close association with the Cytophaga-Flavobacteria-Bacteroides-Taxobacter (CFBT) group. The physiological novelties of these species such as resistance to hydrogen peroxide, desiccation, etc. will be presented. Isolation of microbes that are resistant to hydrogen peroxide has significant implications in the assembly of spacecraft because vapor hydrogen peroxide is the low-heat sterilization technology of choice for spacecraft hardware.

## B12D-0151 1330h POSTER

### Development of Planetary Protection Technology: Bacterial Spore Detection using Lanthanide Luminescence

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The core of bacterial spores contains 1 molar dipicolinic acid, DPA, which can be released into bulk solution by inducing germination with L-alanine or by autoclaving the sample. The released DPA binds terbium, Tb, as a tridentate ligand with high affinity,  $10^9$  M<sup>-1</sup>, which triggers bright green luminescence under UV excitation that can be correlated to a DPA concentration and subsequently to endospore concentration. Current detection limits of this method are at  $10^4$  spores per ml. We will present our current efforts to reach single spore per ml detection limits.

## B12D-0152 1330h POSTER

### Developing Planetary Protection Technology: New Capabilities and Facilities at JPL

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In order to meet planetary protection microbial reduction requirements, in situ life detection and sample return missions will be required to reduce the bioburden levels of their spacecraft to specified levels and then validate that these levels have been met. Use of modern materials and sophisticated electronics and sensors in today's spacecraft dictate the need for cleaning and sterilization technologies capable of reducing bioburden levels while at the same time maintaining material and subassembly integrity. Numerous cleaning and sterilization technologies exist, but their ability to provide biological decontamination on spaceflight hardware needs to be better understood. New laboratory technologies and facilities to support planetary protection are under development at the Jet Propulsion Laboratory. These laboratory capabilities will provide new tools for cleaning, sterilization, and detection and characterization of bioburden on spacecraft. The goal of these activities is to provide new methods to establish and maintain the cleanliness and sterility of NASA hardware before flight. This development combines research in environmental microbiological studies of spacecraft assembly facilities and the development of practical methods for the cleaning and sterilization of hardware. A variety of modern molecular biology, biochemistry, and microscopy approaches are used to evaluate to effectiveness of various methods of cleaning and sterilization.

## B12E MC: Hall D Monday 1330h

### Geophysiology: The Influence of Organisms on Their Geophysical Environment I

Presiding: J Neff, U.S. Geological Survey; G N Cameron, University of Cincinnati

## B12E-0153 1330h POSTER

### Distribution of Dimethylsulfide and Dimethylsulfoniopropionate in Arctic Shelf Region

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Dimethylsulfide (DMS) is the most abundant biogenic sulfur-bearing compounds emitted from ocean to the atmosphere. DMS is oxidized in the atmosphere and condensed as aerosols, which affect the radiative balance of the Earth directly or indirectly. Ice algae are known to accumulate high amount of dimethylsulfoniopropionate (DMSP; precursor of DMS) in their cell for the purpose of cryoprotection. Therefore, the sulfur compound has potential significance in the Arctic climate. Observations in the Arctic Ocean were carried out in September, 1999 and from August to October, 2000. The concentrations of dissolved DMS, dissolved DMSP and particulate DMSP were determined for seawater samples collected at 7-9 water depths within 200 m from the surface at the 21 stations along the Beaufort shelf. Mean concentration of DMS in surface water was about 1.4 nmol/l (Range 0.2-17.5 nmol/l). This suggests that the flux of DMS from this region to the atmosphere is moderate in the observed period. DMS and DMSP appeared within the upper 20 m of the water column, at most of the stations. Concentrations of particulate DMSP were relatively high, though that of dissolved DMSP and DMS were low. This suggests the bacterial consumption of dissolved DMSP and DMS may active in summer. Relatively high concentrations of DMS and DMSP were found at the stations along Barrow Canyon, where the warm water mass was observed. Since the warm water mass in Barrow Canyon is believed to have originated from North Bering Sea, that indicates the Pacific water mass could affect the production of those sulfur compounds. High concentrations of particulate DMSP (maximum 30 nmol/l) were also found at the stations influenced by ice melt water rather than river water. That indicates ice algae are significant producer of DMSP and DMS in Arctic Ocean.

## B12E-0154 1330h POSTER

### Biological albedo reduction of snow and ice on glaciers in Alaska.

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Biogenic contaminants in snow and ice and its effect on surface albedo were investigated on five glaciers in Alaska. Several species of snow algae and dark colored organic material were found in the snow and ice of all of the glaciers. The surface albedo was significantly reduced by red-colored algae (*Chlamydomonas nivalis*) on snow area of the glaciers, and by dark colored material (cryoconite) on ice area. The amounts of snow algae and other biogenic material were different between glaciers: larger amounts of algae and material existed on inland glacier compared to south coastal glaciers of Alaska. The measured surface albedo was lower on the inland glacier than on the south coastal glaciers, consistent with the amount of the biogenic material. Results suggest that the effect of biological activity on surface albedo is more significant on the inland glacier than the south coastal glaciers in Alaska.

URL: <http://www.frontier.iarc.uaf.edu:8080/~nozomu/>

## B12E-0155 1330h POSTER

### Biological Control on Mineral Transformation in Soils ?

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Weathering of primary minerals is commonly linked to biological processes through the production of carbonic and organic acids. Plants can also play a role in weathering by removing soluble constituents and enhancing diffusion gradients within the soil. Here we investigate the synthesis of secondary minerals and the role of plants in removing elements that act as building blocks for these minerals. In order to minimize losses from leaching, we have sampled a chronosequence of soils forming on lava flows on Hawaii Island that receive about 200 mm of rain annually and have never been subjected to high levels of rainfall. The P concentration in the soils drops from almost 3000 mg/kg on a 1.5 ky lava flow to around 1000 mg/kg on a 350